

Therapeutic potential of a combined hepatitis B virus surface and core antigen vaccine in patients with chronic hepatitis B

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Abstract

Purpose The safety and clinical efficacy of a vaccine containing both hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) (HBsAg/HBcAg) were evaluated in patients with chronic hepatitis B (CHB).

Methods Eighteen patients with CHB were administered a vaccine containing 100 µg of HBsAg and 100 µg of HBcAg. The vaccine was administered ten times at 2-weekly intervals, the first five times via the nasal route only and the subsequent five times via both nasal and subcutaneous routes. The safety and efficacy of this

therapeutic approach were assessed by periodic assessment of the patients' general condition, viral kinetics, and biochemical parameters during treatment and 24 and 48 weeks after therapy. The production of cytokines by peripheral blood mononuclear cells (PBMC) and antigen-pulsed dendritic cells (DC) was evaluated to assess the immunomodulatory effects of the HBsAg/HBcAg vaccine in CHB patients.

Results The HBsAg/HBcAg vaccine was safe in all patients. No flare of HBV DNA or alanine aminotransferase (ALT) was recorded in any patient. Sustained HBV DNA negativity and persistently normalized ALT were detected in 9 (50 %) and 18 (100 %) patients with CHB, respectively. PBMC and HBsAg/HBcAg-pulsed DCs from HBsAg/HBcAg-vaccinated CHB patients produced significantly higher levels of various cytokines [interleukin 1β (IL-1β), IL-6, IL-8, IL-12, and tumor necrosis factor α (TNF-α)] than those from control unvaccinated CHB patients ($p < 0.05$) after stimulation with HBsAg/HBcAg in vitro.

Conclusion HBsAg/HBcAg vaccine seems a safe and efficient therapeutic approach for patients with CHB.

Keywords Chronic hepatitis B · Immunotherapy · Therapeutic vaccine · HBsAg/HBcAg vaccine

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Introduction

The therapies available for patients with chronic hepatitis B virus (HBV) infection stimulate both optimism and frustration. Standard interferon (IFN), pegylated IFN, and nucleotide and nucleoside analogs can reduce HBV replication and contain liver damage in some, but not all, patients with chronic hepatitis B (CHB) [1–7]. However,

other studies have shown that antiviral drug treatments may not block HBV-related complications, improve the ultimate clinical outcomes, or positively modulate all intermediate parameters in the majority of CHB patients [8, 9].

These data indicate that there is a pressing need to develop new and novel therapies for CHB patients. HBV is a noncytopathic virus, and studies have suggested that HBV-specific immune responses are weak and narrowly focused in patients chronically infected with HBV compared with those of patients with acute hepatitis B in whom HBV infection is successfully resolved [10, 11].

The importance of host immunity in the pathogenesis of CHB has led to a new field of therapy for CHB patients, in which immunomodulators are used. However, polyclonal immunomodulators, such as cytokines, growth factors, and other nonantigen-specific immune activators other than interferon, have shown either notable adverse effects or insufficient therapeutic efficacy in these patients [12]. A new immunotherapeutic approach to CHB patients emerged in 1994, in which a vaccine containing the hepatitis B surface antigen (HBsAg) is used [13]. In the last 19 years, patients with CHB have been treated with different HBsAg-based vaccines [14], combination therapies of HBsAg-based vaccines and antiviral drugs [15], and HBsAg-pulsed antigen-presenting dendritic cells (DC) [16, 17]. Although vaccine therapies that include an HBsAg-based vaccine are safe in CHB patients, it seems that immunotherapeutic approaches with HBV antigens can be improved in CHB patients by modifying the nature of the vaccine, dose of vaccine, and route of administration.

Evidence has shown that both HBsAg-specific and hepatitis B core antigen (HBcAg)-specific immune responses are required to control HBV replication and to contain liver damage in CHB patients [18]. Direct evidence of the roles of the HBsAg- and HBcAg-specific immune responses was provided by Lau et al., who showed that CHB patients cleared the virus and developed anti-hepatitis B virus (HBV) antibodies after receiving bone-marrow cells from naturally protected donors expressing both HBsAg- and HBcAg-specific immunocytes [19]. However, there is a paucity of information about the safety and efficacy of therapeutic vaccines containing both HBsAg and HBcAg in CHB patients.

In the present study, a therapeutic vaccine containing both HBsAg and HBcAg (HBsAg/HBcAg) was administered to patients with CHB after the safety and efficacy of the HBsAg/HBcAg vaccine had been assessed in normal mice [20], HBV-transgenic mice (a model of the chronic HBV carrier state) [21], and normal healthy human volunteers [22]. In the present study, the HBsAg/HBcAg vaccine was administered via both mucosal (nasal) and subcutaneous routes. We also examined the mechanisms underlying the effects of the HBsAg/HBcAg vaccine by

Table 1 Clinical profiles of the patients

	HBeAg (–)	HBeAg (+)	Total
[A] Study population			
Numbers of patients	11	7	18
Age (years)	31.2 ± 2.8 (20–52) ^a	30.5 ± 2.7 (19–32)	28.3 ± 1.9
Sex (male:female)	9:2	5:2	14:4
Anti-HBe positive	11	0	11
Alanine aminotransferase (IU/l)	69 ± 8 (43–134)	78 ± 13 (54–152)	72 ± 7
HBV DNA (log copies)	4.09 ± 0.26	8.23 ± 1.22	5.54 ± 0.63
HBV genotype			
Genotypes			
Genotype A		1	1
Genotype C	9	3	12
Genotype D	2	3	5
[B] Control subjects			
Numbers of patients	6	4	10
Age (years)	32.6 ± 2.7 (20–44) ^a	29.5 ± 2.6 (20–62)	31.4 ± 1.9
Sex (male:female)	5:1	3:1	8:2
Anti-HBe positive	6	0	6
Alanine aminotransferase (IU/l)	71.6 ± 12.8 (45–130)	79.3 ± 20.2 (56–140)	74.7 ± 7.5
HBV DNA (log copies)	4.1 ± 0.41	8.2 ± 1.58	5.75 ± 0.91
HBV genotype			
Genotypes			
Genotype C	4	2	6
Genotype D	2	2	4

Data are shown as mean and standard error of mean (SEM)

^a Indicates range

assessing the antigen-specific immunomodulation attributable to this therapeutic vaccination in these patients.

Materials and methods

Patients

Twenty patients with CHB were initially enrolled in the study. Two patients withdrew from the clinical trial after the first cycle of vaccination (after receiving five vaccinations by the nasal route). Thus, 18 patients received two cycles of vaccination and were followed up for the designated period of 48 weeks. The diagnosis of CHB was made based on the clinical, virological, and biochemical features of the patients. The clinical details of the patients are shown in Table 1. All of them had HBV DNA in their sera

(5.54 ± 0.63 log copies/ml, $N = 18$), and the levels of alanine aminotransferase (ALT) were above the upper limit of normal (ULN) (72 ± 7 IU/l, $N = 18$) in all patients (Table 1A). None of these patients had received any antiviral or immunomodulatory drugs for the treatment of their HBV infection before their enrollment in this clinical trial.

Ten patients with CHB, with similar levels of HBV DNA and ALT, were enrolled in the study to assess the immunological mechanisms underlying the HBsAg/HBcAg vaccinations. All of them had HBV DNA in their sera (5.75 ± 0.91 log copies/ml, $N = 10$), and the levels of ALT were above the ULN (74 ± 8 IU/l, $N = 10$) in these patients (Table 1B). The magnitudes of the immune responses of the HBsAg/HBcAg-vaccinated CHB patients were compared with those of ten untreated CHB patients (control group).

HBsAg/HBcAg vaccine

The vaccine formulation was designed, produced, and developed by the Center for Genetic Engineering and Biotechnology (Havana, Cuba) and was released as a human-grade product with appropriate quality controls to ensure its sterility, the absence of pyrogens, and the necessary formulation specifications in terms of its composition [20–23]. The vaccine contained *Pichia pastoris*-derived recombinant HBsAg subtype adw2 and purified *Escherichia coli*-expressed recombinant full-length HBcAg (GenBank accession no. X02763). HBsAg was produced as a 22-nm particle as a component of the commercial anti-HBV vaccine, Heberbiovac-HB1 [22]. The HBsAg antigen of this vaccine only comprises the S protein, expressed and purified in a nonglycosylated form.

Vaccination schedule

Therapeutic vaccination was conducted in two cycles (Fig. 1). In the first cycle, 100 µg of HBsAg and 100 µg of HBcAg were administered in a volume of 1.0 ml via the intranasal route using a nasal spray on five occasions at 2-weekly intervals. In the second cycle, the same vaccine formulation was administered simultaneously via the nasal (1.0 ml containing 100 µg of HBsAg and 100 µg of HBcAg) and subcutaneous routes (1.0 ml containing 100 µg of HBsAg and 100 µg of HBcAg) on five occasions at 2-weekly intervals. During the application of the vaccine via the nasal route, the head of the patient was tilted backwards to allow the vaccine to remain in close contact with the nasal mucosa for a long period. The vaccine was administered with a multidose nasal sprayer (VP7D, Valois, France) calibrated to dispense 125 µl with every push on the plunger. Thus, the nasal vaccine was applied

eight times (four in the right nostril and four in the left nostril), and a total of 1.0 ml of vaccine containing 100 µg of HBsAg and 100 µg of HBcAg was delivered.

All patients were observed for 2 h after each vaccination. Serum was collected from each patient before the study commenced and before each vaccination. The post-treatment follow-ups were conducted 24 and 48 weeks after the end of treatment.

Safety evaluation

The occurrence of any adverse reactions to the treatment was one of the main variables assessed in this clinical trial. All adverse events were recorded on the data collection sheets. Adverse reactions were measured immediately and up to 2 h after immunization. Any adverse events during the 2-week interimmunization periods were also recorded before the administration of the next dose of vaccine. In addition to any subjective symptoms, blood from all patients was tested once a fortnight to assess the general parameters of the inflammatory responses, kidney function, and liver function. Sera were also collected 24 and 48 weeks after the treatment was completed to assess the long-term safety of the vaccine therapy.

Efficacy evaluation

The levels of HBV DNA, hepatitis B e antigen (HBeAg), and antibody to HBeAg (anti-HBe) were checked before the commencement of the therapy after the completion of the first cycle of vaccination, after the completion of the second cycle of vaccination and 24 and 48 weeks after the end of the second cycle of vaccinations. ALT levels were measured before the commencement of therapy, once a fortnight during the first and second cycles of vaccination, and again 24 and 48 weeks after the end of the second cycle of vaccination.

Hematological tests

Complete hematological tests were performed to characterize the blood cell populations using conventional procedures. Liver function parameters (albumin, alkaline phosphatase, total protein, platelet, and bilirubin levels) were used to monitor any potential deterioration of the disease during the course of the study. Serum creatinine was measured to assess the kidney function of all patients.

Biochemical, serological, and virological tests

Serum ALT levels and prothrombin time were assessed by a commercial, tertiary-level professional laboratory, as

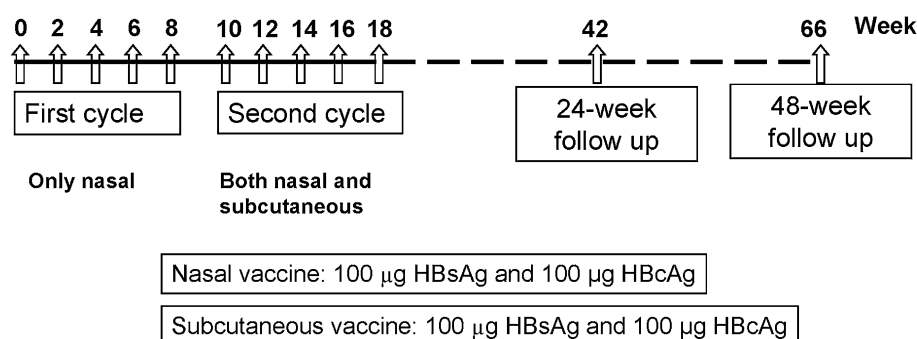


Fig. 1 Immunization protocol for vaccine therapy with a vaccine containing hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) in patients with chronic hepatitis B. Vaccine formulation (1 ml) containing 100 µg of HBsAg and 100 µg of HBcAg was administered to the nasal mucosa on five occasions at 2-weekly intervals (first cycle). Two weeks after the completion of

the first cycle, patients received both nasal vaccinations and subcutaneous vaccinations (second cycle). Safety and efficacy issues were assessed in all patients before vaccination, at all vaccination points during the first and second cycles, and 24 and 48 weeks after the completion of the second cycle of vaccinations

previously described [24]. The cutoff value for abnormal ALT was 42 U/l. HBeAg and anti-HBe were checked with an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Abbott Laboratories, Chicago, IL, USA). Serum HBV DNA was quantified with a polymerase chain reaction (PCR) method using a commercial kit (Amplicor HBV Monitor assay; Roche Molecular Systems, Pleasanton, CA, USA). The detection limit for HBV DNA was 250 copies of HBV DNA/ml.

Immunological assays

Peripheral blood mononuclear cells (PBMCs) and dendritic cells (DCs) were isolated and cytokine production assessed according to previously described methods [25–27]. PBMCs were isolated from fresh heparinized blood using a Ficoll–Hypaque gradient (specific gravity, 1.077). DCs were enriched from an adherent population of PBMCs, as previously described. Briefly, PBMCs were cultured in RPMI 1640 plus 10 % autologous serum, human-grade macrophage-granulocyte colony stimulating factor (800 U/ml), and IL-4 (400 U/ml) (Pepro Tech EC Ltd, London, UK) for 7 days [25]. HBsAg/HBcAg DCs were produced by culturing PBMC-derived DCs with 10 µg of HBsAg/HBcAg for 8 h, as previously described [25, 26]. Pyruvate dehydrogenase complex (PDC; Sigma, St. Louis, MO, USA)-pulsed DCs were also prepared. PBMCs and antigen-pulsed DCs from different patients were cultured for 72 h with or without different combinations of antigens. The levels of different cytokines in the culture supernatants were measured with enzyme-linked immunosorbent assays [27].

Statistical analysis

The patients' profile data are presented as means \pm standard errors of the means (SEM). Cytokine production data

are shown as means \pm standard deviations (SD). One-way analysis of variance, followed by the Tukey–Kramer test as the post hoc test, was performed to analyze the cytokine production data.

Results

Clinical profiles of the patients

The clinical profiles of the patients and controls included in the study are summarized in Table 1A. Seven patients expressed HBeAg, and 11 were negative for HBeAg. All HBeAg-negative patients expressed anti-HBe antibodies. All patients had serum ALT levels above the ULN when enrolled in the study.

Adverse events after vaccination

General

The vaccine was well tolerated, and there was no patient withdrawal in response to adverse events. Two patients discontinued the trial because they became HBV DNA negative and their ALT levels normalized after the first cycle of five nasal vaccinations. The remaining 18 patients were vaccinated in the first and second cycles, and were followed up for 48 weeks after vaccination. Each of these 18 patients attended their physician ten times for vaccinations and twice during the follow-up period. Thus, a total of 216 appearances were recorded, from which we estimated the adverse events during the therapy period. Table 2 shows that in total 35 adverse events were reported.

Table 2 Adverse effects of the vaccine therapy

Adverse effect	Total appearances	Total adverse effects (%)	Total patients	Patients with adverse effects (%)
Pain at injection site	216	25 (11.5)	18	5 (28)
Slight increase in body temperature		4 (1.8)		4 (22)
Indigestion		2 (0.9)		1 (5.6)
Discomfort		4 (1.8)		2 (11.1)

Data in parentheses show the percentages of total adverse effects

Liver and kidney functions

To evaluate any potential impairment of liver function, we assessed the bilirubin values, total proteins, albumin levels, platelet counts, and alkaline phosphatase levels of patients before immunization and after the first and second cycles of vaccine therapy with the HBsAg/HBcAg vaccine. Sera were also collected from all patients 24 and 48 weeks after the end of treatment. No abnormal changes in any liver or kidney function (serum creatinine) parameter were detected in any patient during the follow-up period.

Therapeutic response

Virological response

The virological responses of CHB patients to the vaccine therapy with HBsAg/HBcAg are shown in Fig. 2. After completion of the first cycle (five nasal vaccinations), HBV DNA levels were undetectable (<250 copies/ml) in 5 of 11 HBeAg-negative patients. At the end of the second cycle of vaccinations, HBV DNA was undetectable in six HBeAg-negative patients and one HBeAg-positive patient (Fig. 2). The sera of seven HBeAg-negative patients and two HBeAg-positive CHB patients were negative for HBV DNA 48 weeks after the end of treatment (Fig. 2). A further four HBeAg-positive patients were considered to be partial responders after follow-up for 48 weeks because their serum viral loads had declined by 2–5 log copies (Fig. 2). Six of 12 patients with genotype C and two of five patients with genotype D were HBV DNA negative at the week 48 follow-up.

Biochemical response

No flare in ALT was detected during the immunizations or during follow-up in any patient. The kinetics of ALT at the

five observation points are shown in Table 3. Fluctuations in ALT were seen at some points between the start of therapy and the end of the first and second cycles of vaccination. However, ALT was below ULN in 16 patients at the end of the second cycle and in all patients 48 weeks after the end of treatment.

Serological response

HBeAg was eliminated from the blood in three of seven HBeAg-positive patients at the end of the treatment, and this response was sustained at the 48-week follow-up. Two of these patients also developed anti-HBe antibodies in their sera. HBV DNA was undetectable in both of these patients.

Immunological response

To estimate the spontaneous production of cytokines, PBMCs from patients who had received five nasal vaccinations were stimulated with HBsAg/HBcAg or PDC, an irrelevant antigen, or were not stimulated with any activator. As shown in Fig. 3, the levels of IL-1 β , IL-6, IL-12, TNF- α , and IL-8 were significantly higher in the culture supernatants from the HBsAg/HBcAg-stimulated PBMC than in those stimulated with PDC or with no antigen (spontaneous).

DCs were then isolated from five patients with CHB after the first and second cycles of vaccination. DCs were also isolated from unvaccinated control patients with CHB. HBsAg/HBcAg-pulsed DCs produced significantly higher levels of IL-1 β , TNF- α , and IL-12 than PDC-pulsed DCs from HBsAg/HBcAg-vaccinated CHB patients or HBsAg/HBcAg-pulsed DCs from control CHB patients ($p < 0.05$) (Fig. 4).

Discussion

The present study demonstrates the safety and efficacy of a vaccine formulation containing both HBsAg and HBcAg in the treatment of patients with CHB. The safety and efficacy profiles of the HBsAg/HBcAg-based therapeutic vaccine against CHB allow considerable optimism when compared with those of current treatment modalities [1–7].

There were no immediate or delayed adverse effects of this therapy. Vaccination induced HBV DNA negativity and normalized ALT, and these effects were sustained for 48 weeks after the cessation of therapy in about 50 % of CHB patients. When these factors are considered collectively, the HBsAg/HBcAg vaccine represents a safe therapeutic regimen of finite duration for CHB patients. Vaccine therapy with the HBsAg/HBcAg vaccine was

Fig. 2 HBV DNA kinetics in the sera of patients with chronic hepatitis B after treatment with a vaccine containing hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) (HBsAg/HBcAg). The vaccine was given as described in Fig. 1. **a** Virological effects of vaccine therapy in HBeAg-positive patients. **b** Effects of vaccine therapy in HBeAg-negative patients

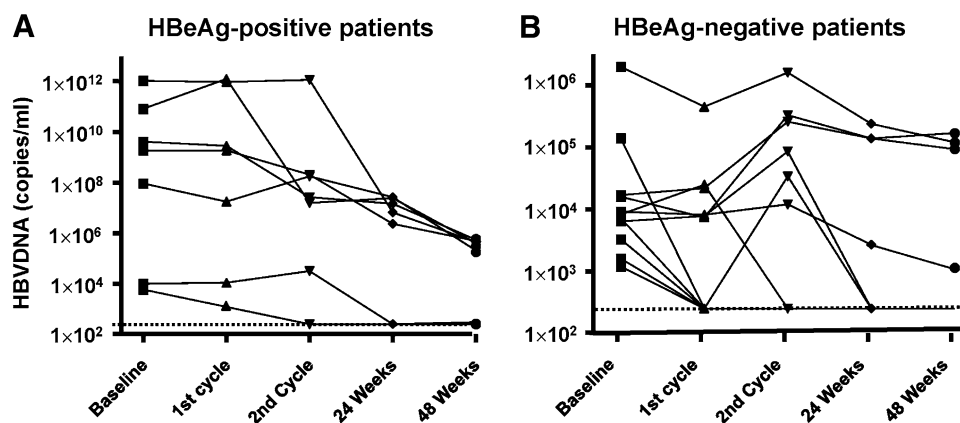


Table 3 Kinetics of alanine aminotransferase in patients with chronic hepatitis B

Patients No.	Base line	1st cycle	2nd cycle	24-week	48-week
1	152 ^a	22	21	24	21
2	68	28	20	31	27
3	68	33	24	29	23
4	63	43	28	21	31
5	74	44	30	28	30
6	54	27	21	25	25
7	67	55	59	45	32
8	43	25	20	24	30
9	49	25	30	28	24
10	48	90	20	27	31
11	134	24	67	43	29
12	47	65	28	29	21
13	54	21	20	25	20
14	86	43	41	37	24
15	66	27	22	32	28
16	67	21	22	25	31
17	78	26	20	29	26
18	82	24	20	31	32

^a The levels of alanine aminotransferase have been shown as IU/l

more effective in HBeAg-negative patients than in HBeAg-positive patients. However, HBV DNA negativity and HBeAg seroconversion were also seen in some patients with HBeAg-positive CHB. Moreover, all HBeAg-positive patients showed a considerable reduction in HBV DNA and sustained ALT normalization.

Another novel finding of the present study is that this vaccine confers antiviral and liver-protecting effects when administered via the nasal route, with no nasal irritation or damage to the nasal mucosa. Data collected at the end of the first cycle of vaccinations, which involved only five nasal vaccinations, showed HBV DNA negativity in 6 patients and ALT normalization in 12 patients. However, these patients received a second cycle of vaccination via

both the nasal and subcutaneous routes, so the real potential therapeutic efficacy of the nasal vaccination alone was not ascertained in the present study. If the long-term efficacy of therapeutic HBsAg/HBcAg vaccinations administered via the nasal route can be confirmed in future studies, a new dimension of immunotherapy with this vaccine will be available in developing countries in various parts of Asia and Africa.

Different therapeutic vaccination regimens, including HBsAg-based vaccines [13, 14], combinations of HBsAg vaccines and antiviral drugs [15], and cell-based vaccines [16, 17], have been used to treat CHB patients. These immunotherapeutic strategies have been shown to be safe, but they do not sustainably control HBV replication or contain liver damage. Maini et al. [18] have provided credible evidence that increased levels of intrahepatic HBcAg-specific cytotoxic T cells (CTL) may be related to the containment of HBV replication and liver damage in CHB patients. The present study presents translational research in which the clinical application of basic knowledge may facilitate the containment of HBV and control liver damage in CHB patients.

There are some limitations to this study. For example, the sample size was small. Considerable number of HBeAg-negative patients have low HBV DNA loads. The vaccination protocol (both nasal and parenteral) was arbitrarily selected. The long-term effects of therapeutic vaccines also remain to be assessed in a randomized controlled trial.

There are several possible options for improving this vaccination protocol. The doses of the antigens and the frequency of the vaccination may be increased in future trials. Monotherapy with the HBsAg/HBcAg vaccine was assessed in the present study, but a vaccine therapy can be applied before, after, or concurrently with potent antiviral drugs directed against HBV. The real impact of the second cycle of vaccination should also be evaluated in more detail. Increased cytokine production by DC was attributed to the second cycle of vaccination, but the levels of cytokines did

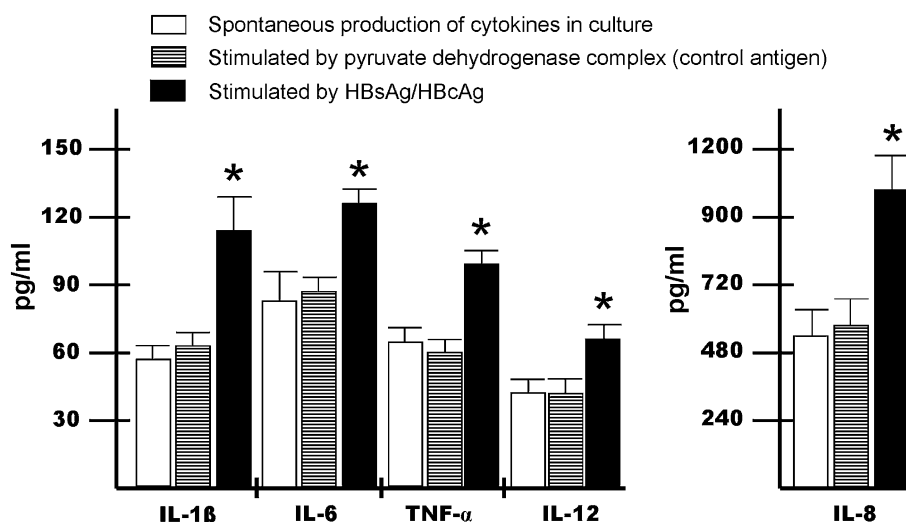


Fig. 3 Antigen-induced production of proinflammatory cytokines by the peripheral blood mononuclear cells (PBMCs) of patients at the end of the first cycle of vaccination (five nasal vaccinations at 2-weekly intervals). PBMCs of patients with chronic hepatitis B were stimulated with HBsAg/HBcAg or pyruvate dehydrogenase (PDC), an

irrelevant antigen, or were unstimulated (to assess the spontaneous production of cytokines). Data shown are means \pm standard deviations (SD). * $p < 0.05$ compared with PDC stimulation or spontaneous production

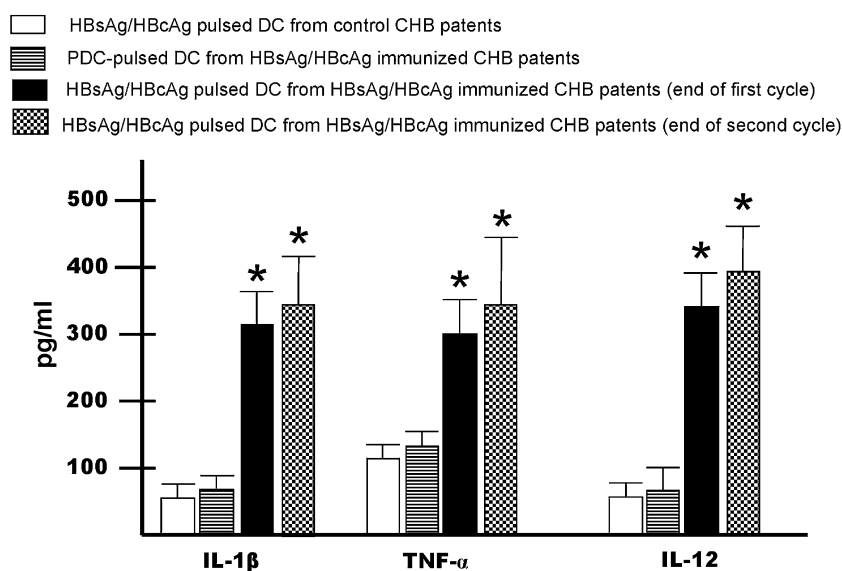


Fig. 4 Increased production of cytokines by HBsAg/HBcAg-pulsed dendritic cells (DC) from patients after the first and second cycles of vaccination compared with PDC-pulsed DCs from HBsAg/HBcAg-vaccinated CHB patients or HBsAg/HBcAg-pulsed DCs from control CHB patients. The DCs of different patients were pulsed with HBsAg/HBcAg or PDC at different times. The levels of IL-1β,

TNF-α, and IL-12 were measured in the culture supernatants. * $p < 0.05$ compared with the HBsAg/HBcAg-pulsed DCs of unvaccinated control CHB patients or PDC-pulsed DCs of HBsAg/HBcAg-vaccinated CHB patients. Data shown are means \pm standard deviations (SD)

not differ significantly between the first and second cycles (Fig. 4). The relative immunomodulatory capacities of HBsAg, HBcAg, and HBsAg/HBcAg vaccines in CHB patients were not clarified in the present study. However, we have shown that HBcAg and HBsAg/HBcAg are more immunogenic than HBsAg in HBV transgenic mice, a model of the chronic HBV carrier state [21, 28], although this

remains to be confirmed in patients with CHB. To increase our insight, we have begun a phase III study of this vaccine in 75 patients, with a control arm of 76 patients with CHB who will receive pegylated interferon (registered at ClinicalTrials.gov: NCT01374308).

Extensive evaluation of the mechanism of action of this vaccine therapy could not be addressed properly in the

present study. However, we have shown that the HBsAg/HBcAg vaccination induced significantly higher levels of (1) proinflammatory cytokines and (2) antigen-presenting DC activation in the peripheral blood of CHB patients. Further research into the HBsAg/HBcAg-induced activation of hepatic immunocytes is required to better understand the mechanism of action of the HBsAg/HBcAg vaccine in CHB patients, especially with an assessment of antigen-specific CTL in the liver.

The mucosal routes of therapeutic vaccination have been poorly studied. However, most immunocytes (about 80 %) are associated with mucosal compartments, where they are poorly stimulated after parenteral immunization because of the compartmentalization of the immune system [29]. In the present study, we found that the vaccination of CHB patients with HBsAg/HBcAg via the nasal route induced increased proinflammatory cytokines and the activation of DCs (Figs. 3, 4). We have previously shown that both systemic and mucosal immune responses are induced by nasal vaccination in mice [23]. Studies in human immunodeficiency virus patients have also shown that the stimulation of mucosal immunity is useful in the setting of concomitant systemic immunosuppression [30].

In summary, this HBsAg/HBcAg-based vaccine provides an evidence-based immunotherapy for CHB patients. The vaccine therapy was safe in all patients, and HBV DNA negativity and sustained normalization of ALT were detected in 50 % of patients. A phase III study of this vaccine is underway to confirm the findings of this pilot study. There are ample opportunities to improve the design of this therapeutic vaccine, so further studies of it should be undertaken in different parts of the world.

Compliance with Ethical Requirements All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, and Farabi Hospital, Dhaka, Bangladesh, and with the Declaration of Helsinki 1975, as revised in 2008. Informed consent was obtained from all patients for their inclusion in the study.

Conflict of interest Mamun Al-Mahtab, Sheikh Mohammad Fazle Akbar, Julio Cesar Aguilar, MD, Helal Uddin, MD, Sakirul Islam Khan, and Salimur Rahman declare that they have no conflicts of interest.

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